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Mosquito larvicidal and pupicidal activity of *Euphorbia hirta* Linn. (Family: Euphorbiaceae) and *Bacillus sphaericus* against *Anopheles stephensi* Liston. (Diptera: Culicidae)

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ABSTRACT

Objective: To explore the larvicidal and pupicidal activity of *Euphorbia hirta (E. hirta)* leaf extract and *Bacillus sphaericus (B. sphaericus)* against the malarial vector, *Anopheles stephensi (An. stephensi)*. **Methods:** The larvicidal and pupicidal activity was assayed against *An. stephensi* at various concentrations ranging from (75-375 ppm) under the laboratory as well as field conditions. The LC_{50} and LC_{90} value of the *E. hirta* leaf extract was determined by probit analysis. **Results:** The plant extract showed larvicidal effects after 24 h of exposure; however, the highest larval mortality was found in the methanol extract of *E. hirta* against the first to fourth instars larvae and pupae of values LC_{50}= 137.40, 172.65, 217.81, 269.37 and 332.39 ppm; *B. sphaericus* against the first to fourth instars larvae and pupae of values LC_{50}= 44.29, 55.83, 68.51, 82.19 and 95.55 ppm, respectively. Moreover, combined treatment of values of LC_{50}= 79.13, 80.42, 86.01, 93.00 and 98.12 ppm, respectively. No mortality was observed in the control. **Conclusions:** These results suggest methanol leaf extracts of *E. hirta* and *B. sphaericus* have potential to be used as an ideal eco-friendly approach for the control of the malarial vector, *An. stephensi* as target species of vector control programs. This study provides the first report on the combined mosquito larvicidal and pupicidal activity of this plant crude extract and bacterial toxin against *An. stephensi* mosquitoes.

1. Introduction

Malaria is one of the serious scourges inflicted upon humanity. It causes human mortality and morbidity along with great financial loss. Almost all tropical regions of the world are experiencing the resurgence and reoccurrence of one of the world's most deadly diseases, *ie.* malaria, and India is no exception. In the Indian scenario, almost the entire country is endemic to the disease due to favorable ecological conditions. The incidence of malaria in the country is largely erratic regionally because of various biological and climatic factors. Further, malaria exerts an enormous toll in terms of medical cost and in days of labor cost in the country. Besides the use of antimalarial drugs, malaria control in the developing countries is based largely on vector eradication by the application of mosquito larvicides as an ideal method for controlling mosquito infestation with malaria parasites. Among 53 anopheline species present in India, nine are vectors malaria. *Anopheles stephensi* (*An. stephensi*) is responsible for transmission of malaria in urban regions of India[1].

An obvious method for the control of mosquito-borne diseases is the use of insecticides, and many synthetic agents have been developed and employed in the field with considerable success. However, one major drawback with the use of chemical insecticides is that they are non-selective and could be harmful to other organisms in the environment. It has also provoked undesirable effects, including toxicity to nontarget organisms, and fostered environmental and human health concerns[2]. The toxicity problem, together with the growing incidence of insect resistance, has called attention to the need for novel insecticides[3] and for more detailed studies of naturally

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occurring insecticides[4]. These problems have highlighted the need for the development of new strategies for selective mosquito larval control. Extracts or essential oils from plants may be alternative sources of mosquito larval control agents, as they constitute a rich source of bioactive compounds that are biodegradable into nontoxic products and potentially suitable for use in control of mosquito larvae. In fact, many researchers have reported on the effectiveness of plant extracts or essential oils against mosquito larvae[5-7].

Euphorbia hirta (E. hirta) belongs to the family Euphorbiaceae. It is a small annual herb common to tropical countries. It is usually erect, slender-stemmed; spreading up to 80 cm tall, though sometimes it can be seen lying down. The plant is an annual broad-leaved herb that has a hairy stem with many branches from the base to top. The leaves are opposite, elliptical, oblong or oblong-lanceolate, with a faintly toothed margin and darker on the upper surface. The flower are small, numerous and crowded together in dense cymes (dense clusters in upper axils) about 1 cm in diameter. The stem and leaves produce a white or milky juice when cut. It is frequently seen occupying open waste spaces, banks of watercourses, grasslands, road sides, and pathways[8,9].

The genus *Euphorbia* (Euphorbiaceae) is chemically defined by the occurrence of a large number of polyfunctional diterpenoids with the tigliane (phorbol), ingenane, and daphnane skeletons[10]; lectins and lysozymes with recognized biological properties[11,12]. Most of these are skin irritants and toxic; in addition, many of them are skin tumor promoters. Nonirritant polyfunctional macrocyclic diterpenoids with the lathyrane and jatrophane skeletons have also been isolated from the *Euphorbia* species. The plant has been reported to contain quercitrin[13] and polyphenols[14]. The extracts were reported as anthelmintic[15], repellent, antifeedant and controlling *Plutella xylostella*[16] and *Rotylenchulus reniformis*[17], antimicrobial[18] antibacterial, and against worms[19].

Many studies on plant extracts against mosquito larvae have been conducted around the world. Larvicidal activity of *Gliricidia sepium* crude ethanol extracts of dried leaves, fresh leaves, dried petioles and stem bark were tested for their activities against third instar larvae of *An. stephensi*, *Anopheles aegypti* and *Culex quinquefasciatus*[20]. Studies were focused on the effect of some indigenous plants on the larvicide and ovipositional properties on *An. stephensi*[21]. The petroleum ether root extract of *Solanum xanthocarpum* extract was observed to be toxic against the larvae of *An. stephensi*[22]; the leaf extract of *Solanum trilobatum* was tested under laboratory conditions for oviposition-deterrent and skin-repellent activities against *An. stephensi*[23].

Bacillus sphaericus (B. sphaericus) is a naturally occurring soil bacterium that can effectively kill mosquito larvae present in water. *B. sphaericus* has the unique property of being able to control mosquito larvae in water that is rich in organic matter. *B. sphaericus* is effective against *Culex* spp. but is less effective against some other mosquito species. Commercially available formulations of *B. sphaericus* are sold under the trade name Vectolex. When community mosquito control is needed to reduce mosquito-borne disease, the Department of Health favors the use of larvicide applications targeted to the breeding source of mosquitoes[24]. It produces a round spore in the terminal portion of its cell. Two types of proteins, crystal toxins and Mtx toxins, produce the larvicidal effect by acting on specific receptors in the midgut of culicid larvae, causing a lethal cytopathological effect. Despite its high toxicity, *B. sphaericus* is very specific, affecting only a small number of susceptible species. Another important factor is the bioinsecticide's capacity to recycle itself in dead Culicidae larvae, resulting in greater persistence and greater larvicidal effect by the product. Based on such characteristics, *B. sphaericus* bioinsecticides are indicated for combating lymphatic filariasis, West Nile fever, and malaria among other diseases.

Many biological control agents have been evaluated against larval stages of mosquitoes, of which the most successful ones comprise bacteria such as *Bacillus thuringiensis (B. thuringiensis)* and *B. sphaericus*[25]. The use of *B. sphaericus* as a potential biolarvicides in India is limited due to the development of resistance by the target mosquito species[26]. Well-known bacterial agents which have been used successfully for mosquito control are *B. thuringiensis* and *B. sphaericus*. Two bacterial agents such as the *B. thuringiensis* and *B. sphaericus* are being widely used for control of mosquito breeding in a variety of habitats[27-31]. The mosquitocidal activity of the highly active strain of *B. sphaericus* resulted in their development as a commercial larvicides. This is now used in many countries in various parts of the world to control vector and nuisance mosquito species[32].

The present investigation was to explore the mosquito control agent under laboratory as well as field conditions. The plant extracts and *B. sphaericus* are reported to have mosquitocidal properties against malarial vector, *An. stephensi* as target species.

2. Materials and methods

2.1. Collection of eggs and maintenance of larvae

The eggs of *An. stephensi* were collected from National Centre for Disease Control field station of Mettupalayam, Tamil Nadu, India, using an "O"-type brush. These eggs were brought to the laboratory and transferred to 18 cm× 13 cm×4 cm enamel trays containing 500 mL of water for hatching. The mosquito larvae were pedigree dog biscuits and yeast at 3:1 ratio. The feeding was continued until the larvae transformed into the pupal stage.

2.2. Maintenance of pupae and adults

The pupae were collected from the culture trays and transferred to plastic containers (12 cm×12 cm) containing

500 mL of water with the help of a dipper. The plastic jars were kept in a 90 cm× 90 cm× 90 cm mosquito cage for adult emergence. Mosquito larvae were maintained at (27 ±2) ℃, 75%-85% relative humidity, under a photoperiod of 14:10 (light/dark). A 10% sugar solution was provided for a period of 3 days before blood feeding.

2.3. Blood feeding of adult An. stephensi

The adult female mosquitoes were allowed to feed on the blood of a rabbit (a rabbit per day, exposed on the dorsal side) for 2 days, to ensure adequate blood feeding for 5 days. After blood feeding, enamel trays with water from the culture trays were placed in the cage as oviposition substrates.

2.4. Collection of plant and preparation of extract

The *E. hirta* plants were collected from in and around Bharathiar University Campus, Coimbatore. The plants were identified at Botanical Survey of India, Coimbatore, India. *E. hirta* leaves were washed with tap water and shade dried at room temperature. The dried plant materials (leaves) were powdered by an electrical blender. From the powder 500 g of the plant material were extracted with 1.5 L of organic solvents of methanol for using a Soxhlet apparatus boiling point 60-80 ℃ for h[33]. The extracts were filtered through a Buchner funnel with Whatman number 1 filter paper. The crude plant extracts were evaporated to dryness in rotary vacuum evaporator. One gram of the plant residue was dissolved in 100 mL of acetone (stock solution) considered as 1% stock solution. From this stock solution concentrations were prepared ranging from75, 150, 225, 300 and 375 ppm, respectively.

2.5. Microbial bioassay

B. sphaericus was obtained from T- Stanes & Company Limited, Research and Development Coimbatore, Tamil Nadu, India. The organism was grown in a liquid medium containing (in grams per liter of distilled water): $FeSO_4 \cdot 7H_2O$, 0.01; $MnSO_4$, 0.1; $MgSO_4 \cdot 7H_2O$, 0.2; $CaCl_2$, 0.08; K_2HPO_4, 0.025; yeast extract, 2; peptone, 4; and D-glucose, 1 and casein, 5. Solutions of yeast extract, peptone casein, D-glucose, K_2HPO_4 and $CaCl_2$ were separately prepared, sterilized, and added before inoculation. The pH of the medium was adjusted to 7.1 before sterilization. The required quantity of *B. sphaericus* was thoroughly mixed with distilled water and prepared at various concentrations ranging from 10, 20, 40, 60 and 80 ppm, respectively.

2.6. Larval/pupal toxicity test

Laboratory colonies of mosquito larvae/pupae were used for the larvicidal/pupicidal activity. Twenty-five numbers of first to fourth instars larvae and pupae were introduce into 500 mL glass beaker containing 249 mL of de-chlorinated water and 1 mL of desired concentrations of plant extract and *B. sphaericus* were added. Larval food was given for the test larvae. At each tested concentration two to five trials were made and each trial consisted of five replicates. The control was setup by mixing 1 mL of acetone with 249 mL of dechlorinated water. The larvae and pupae were exposed to dechlorinated water without acetone served as control. The control mortalities were corrected by using Abbott's formula[34]:

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Control mortality}} \times 100$$

$$\text{Percented mortality} = \frac{\text{Number of dead larvae/pupae}}{\text{Number of larvae/pupae introduced}} \times 100$$

The LC_{50} and LC_{90} were calculated from toxicity data by using probit analysis[35].

2.7. Field trail

For the field trial, the quantity of plant extract residues and *B. sphaericus* (Bs) required (based on laboratory LC_{50} and LC_{90} values) quantity for each treatment was determined by calculating the total surface area of drinking water bodies in each habitat. The required quantities of *E. hirta* and Bs were mixed thoroughly with water in a bucket with constant agitation. Teepol was used as emulsifying agent (0.05%). Field applications of the *E. hirta* leaf extracts and Bs were done with the help of a knapsack sprayer (Sujatha Products, India, Private Limited, 2010) and uniformly on the surface of the drinking water bodies in each habitat. Dipper sampling and counting of larvae monitored the larval density before 24, 48 and 72 h after the treatment. A separate sample was taken to determine the composition of each larval habitat. Six trails were conducted for *E. hirta* of the plant extracts and *B. sphaericus* alone and combined the treatment.

The percentage of reduction was calculated by the following formula:

$$\text{Percentage of reduction} = \frac{C - T}{C} \times 100$$

Where C is the total number of mosquitoes in control, T is the total number of mosquitoes in treatment.

2.8. Statistical analysis

All data were subjected to analysis of variance; the means were separated using Duncan's multiple range tests by Alder and Rossler[36]. The average larval mortality data were subjected to probit analysis for calculating, LC_{50}, LC_{90} and other statistics at 95% confidence limits of upper confidence limit (UCL) and lower confidence limit (LCL) and chi-square values calculated using the SPSS 16.0 version (Statistical software package). The values were expressed

as mean±standard deviation of five replicates. Results with $P<0.05$ were considered to be statistically significant.

3. Results

Larval and pupal mortality of *An. stephensi* after the treatment of methanol extract of *E. hirta* leaf extract was observed. Table 1 illustrates the larval and pupal mortality of *An. stephensi* (Ⅰ to Ⅳ instars) after the treatment of *E. hirta* at different concentrations (75 to 375 ppm). 40.8% mortality was noted at 1st instar larvae by the treatment of *E. hirta* at 75 ppm, whereas it has been increased to 81.6% at 375 ppm of *E. hirta* leaf extract treatment. Similar trend has been noted for all the instars of *An. stephensi* at different concentration of *E. hirta* treatment. The LC_{50} and LC_{90} values were represented as follows: LC_{50} value of 1st instar was 137.40 ppm, 2nd instar was 172.65 ppm, 3rd instar was 217.81 ppm, and 4th instar was 269.37 ppm, respectively. The LC_{90} value of 1st instar was 470.69 ppm, 2nd instar was 531.43 ppm, 3rd instar was 590.77 ppm, and 4th instar was 685.60 ppm, respectively. The LC_{50} value of pupae was 332.39 ppm, and the LC_{90} value of pupae was 779.80 ppm, respectively.

Table 2 shows the larval mortality of *An. stephensi* (Ⅰ to Ⅳ instars) after the treatment of *B. sphaericus* at different concentrations (10 to 80 ppm). Thirty two percent mortality was noted at 1st instar larvae by the treatment of *B. sphaericus* at 10 ppm, whereas it has been increased to 69.4% at 80 ppm of *B. sphaericus* treatment. Similar trends have been noted for all the instars of *An. stephensi* at different concentrations. The LC_{50} and LC_{90} values were represented as follows: the LC_{50} value of 1st instar was 44.29 ppm, 2nd instar was 55.83 ppm, 3rd instar was 68.51 ppm, and 4th instar was 82.19 ppm, and pupa was 95.55 ppm, respectively. The LC_{90} value of 1st instar was 138.27 ppm, 2nd instar was 165.17 ppm, 3rd instar was 178.30 ppm, and 4th instar was 199.17 ppm, and pupa was 213.06 ppm, respectively.

Table 3 provides the combined larval mortality after treatment of EHLE and *B. sphaericus* for all the larval instars. The concentration at 75+40 combined EHLE + *B. sphaericus* treatment for 4th instar larval mortality was 69.2%. LC_{50} and LC_{90} values were represented as follows: LC_{50} value

Table 1
Larval and pupal toxicity effect of *E. hirta* against malarial vector, *An. stephensi*.

Mosquito larval instars and pupa	% of Larval and pupal mortality ± SD					LC_{50} (LCL– UCL)	LC_{90} (LCL – UCL)	$x^2(df=4)$
	75 ppm	150 ppm	225 ppm	300 ppm	375 ppm			
1st instar	40.8±0.7	51.2±1.3	63.4±1.0	74.0±2.0	81.6±1.8	137.40(90.57–170.06)	470.69(407.32–581.00)	0.05*
2nd instar	36.0±1.4	47.2±1.3	58.4±1.0	65.8±1.7	77.2±0.7	172.65(130.92–204.72)	531.43(453.33–673.66)	0.21*
3rd instar	31.2±1.1	40.4±1.3	52.8±1.7	58.6±1.4	71.6±1.6	217.81(182.54–251.67)	590.77(498.56–763.33)	0.45*
4th instar	27.2±1.7	36.0±1.4	45.6±1.8	51.8±1.6	63.6±2.0	269.37(232.85–317.28)	685.60(563.16–934.94)	0.23*
Pupa	22.6±1.3	30.4±1.0	39.0±1.4	45.0±0.6	55.2±0.7	332.39(287.54–410.88)	779.80(625.52–1117.81)	0.13*

Control–Nil mortality, LCL – Lower confidence Limit, UCL – Upper confidence Limit, x^2 – *Chi*–square value, *df* – degrees of freedom.
*Significant at $P < 0.05$ level.

Table 2
Larval and pupal toxicity effect of *B. sphaericus* against malarial vector, *An. stephensi*.

Mosquito larval instars and pupa	% of Larval and pupal mortality ± SD					LC_{50} (LCL– UCL)	LC_{90} (LCL – UCL)	$x^2(df=4)$
	10 ppm	20 ppm	40 ppm	60 ppm	80 ppm			
1st Instar	32.0±1.2	38.2±1.6	46.0±1.4	58.2±0.9	69.4±1.0	44.29(35.82–53.33)	138.27(113.53–185.90)	0.19*
2nd Instar	29.2±0.7	35.0±0.6	41.0±1.4	52.6±1.8	61.2±1.7	55.83(46.17–69.82)	165.17(130.71–240.01)	0.20*
3rd Instar	25.0±1.4	29.2±1.1	36.4±1.0	44.2±1.3	56.8±1.7	68.51(57.42–88.10)	178.30(140.17–262.35)	0.26*
4th Instar	21.8±1.1	26.4±1.0	31.2±0.7	36.0±1.4	52.4±1.8	82.19(67.83–112.08)	199.17(153.10–308.48)	1.44*
Pupa	18.2±1.1	21.6±0.8	26.0±1.4	31.4±1.0	46.2±0.7	95.55(77.86–135.38)	213.06(162.21–337.40)	1.07*

Control–Nil mortality, LCL – Lower confidence Limit, UCL – Upper confidence Limit, x^2 – *Chi*–square value, *df* – degrees of freedom.
*Significant at $P < 0.05$ level.

Table 3
Combined effect of larval and pupal mortality of methanol extract of *E. hirta* and *B. sphaericus* against malarial vector, *An. stephensi*.

Mosquito larval instars and pupa	% of Larval and pupal mortality ± SD					LC_{50} (LCL– UCL)	LC_{90} (LCL – UCL)	$x^2(df=4)$
	75 +5	75 + 10	75 + 20	75 +30	75 + 40			
1st Instar	52.4±1.8	66.8±1.9	72.2±1.6	88.6±1.3	99.6±0.4	79.13(59.07–86.14)	104.26(96.92–126.35)	7.15*
2nd Instar	44.2±1.7	63.8±1.9	67.4±1.3	83.0±1.4	88.4±1.3	80.42(74.14–84.55)	115.91(110.24–125.23)	3.85*
3rd Instar	39.0±1.4	53.6±1.9	60.4±1.0	69.8±0.7	77.0±1.6	86.01(79.17– 90.51)	133.75(123.32– 154.12)	1.85*
4th Instar	31.2±2.3	46.6±1.0	56.2±0.7	61.0±1.4	69.2±1.1	93.00(87.71– 97.50)	144.02(130.85– 170.95)	3.28*
Pupa	28.4±1.3	39.6±1.0	52.6±1.6	56.2±1.4	63.4±1.8	98.12(93.37– 103.50)	151.34(136.01– 183.77)	2.69*

Control–Nil mortality, LCL – Lower confidence Limit, UCL – Upper confidence Limit, x^2 – *Chi*–square value, *df* – degrees of freedom.
*Significant at $P < 0.05$ level.

of 1st instar was 79.13%, 2nd instar was 80.42%, 3rd instar was 86.01% and 4th instar was 93.00%, respectively. The LC_{90} value of 1st instar was 104.26 ppm, Ⅱ instar was 115.91 ppm, Ⅲ instar was 133.75 ppm, and Ⅳ instar was 144.02 ppm, respectively. The χ^2 values are significant at $P<0.05$ level. The 95% confidence limits LC_{50}, LC_{90} (LCL-UCL) values were also calculated. Larval and pupal mortality was observed after 24 h exposure. No mortality was observed in the control group.

Total number 425 *An. stephensi* larvae found were observed in the drinking water body systems. After treated with *E. hirta* against *An. stephensi* larval density was reduced by 13.17%, 37.64% and 84.00% at 24, 48 and 72 h, respectively. Similarly, the reductions of *An. stephensi* larval densities after treatment with *B. sphaericus* were 8.47%, 29.41% and 79.52%, respectively. Combined effect of *E. hirta* and *B. sphaericus* were 44.23%, 81.64% and 100.0% at 24, 48 and 72 h, respectively (Table 4, 5).

Table 4
Field trail by using plant extracts of *E. hirta* and *B. sphaericus* drinking water tanks against *An. stephensi.*

Sample No.	Before treatment	After treatment					
		E. hirta			*B. sphaericus*		
		24 h	48 h	72 h	24 h	48 h	72 h
1	85	66	44	15	79	52	20
2	75	69	59	13	68	72	18
3	49	37	29	7	44	34	9
4	66	59	48	12	58	52	20
5	96	89	44	11	90	47	16
6	54	49	41	10	50	43	14
Average	70.8	61.5	44.1	11.3	64.8	50.0	14.5
Total	425	369	265	68	389	300	87

Table 5
Field trail by using combined effect of drinking water tanks 0.5×0.5×1.0 against *An. stephensi.*

Sample No.	Before treatment	After treatment		
		24 h	48 h	72 h
1	85	55	12	–
2	75	42	11	–
3	49	28	9	–
4	66	30	15	–
5	96	51	22	–
6	54	31	9	–
Average	70.8	39.5	13.0	0.0
Total	425	237	78	0

4. Discussion

Malaria is one of the most common vector-borne diseases widespread in tropical and subtropical regions, including parts of the America, Asia, and Africa[37]. Malaria is the world's most dreadful tropical disease. Mosquito-borne diseases are endemic in more than over 100 countries, causing mortality of nearly two million people every year, and at least one million children die of such diseases each year, leaving as many as 2 100 million people at risk around the world[38]. The secondary metabolite of plant origins makes up a vast repository compounds with a wide range of biological activities. There have been many reports of higher plant extracts possessing relatively good potential to inhibit viruses[39]. The methanol extract of *Spheranthus indicus* showed macro filaricidal activity by worm motility and subsequent mortality was observed[40]. The latex of *Calotropis procera* has shown larvicidal efficacy against all three important vector species, *Aedes aegypti*, *An. stephensi* and *Culex quinquefasciatus* in India[41].

The direct and indirect contributions of such effects to treatment efficacy through reduced larval feeding and fitness need to be properly understood in order to improve the use of botanical insecticides for of *An. stephensi*. These and other naturally occurring insecticides may play a more prominent role in mosquito control programs in the future[42]. David *et al* found that phytochemicals primarily affect the midgut epithelium and secondarily affect the gastric caeca and the malpighian tubules in mosquito larvae[43]. Furthermore, the crude extracts may be more effective compared to the individual active compounds, due to natural synergism that discourages the development of resistance in the vectors[44]. The leaf methanol extract of *Cassia fistula* was tested for larvicidal and ovicidal activity of against *Culex quinquefasciatus* and *An. stephensi*, with the LC_{50} values of 17.97 and 20.57 mg/L, respectively[45].

The crude and column chromatographic fractions of the methanol leaf extract of *Jatropha curcas* were tested for their larvicidal activities against the laboratory-reared late third instar larvae of *Anopheles arabiensis* [46]. Crude extract of flower, leaf, and stem of *Spilanthes acmella* L. plants were tried against *An. stephensi* Liston and found that the LC_{50} and LC_{90} values of flower extract were more than the leaf and stem extracts against *An. stephensi*[47]. The crude petroleum ether leaf extract of *Jatropha curcas* to have larvicidal activity with the LC_{50} of <100 ppm on the early fourth instar larvae of vector mosquitoes including *Culex quinquefasciatus*, *An. stephensi*, and *Aedes aegypti* [48].

Larvicidal activities of ethanol extract of *Allium sativum* (garlic bulb) against the filarial vector, *Culex quinquefasciatus* with the LC_{50} values for the second, third and fourth larval instars were 144.54, 165.70 and 184.18 ppm, respectively. The results obtained show that this plant material exhibited significant activity and could be considered as potent natural larvicidal agent[49]. *Lippia citriodora* essential oil exhibited an LC_{50} value of 101.4 ppm against the third instars larvae of *An. stephensi*[50]. The larvicidal and pupicidal efficacy of *Solanum xanthocarpum* leaf extract with LC_{50} value of first to fourth instars larvae and pupae 155.29, 198.32, 271.12, 377.44 and 448.41 ppm, respectively. The LC_{90} value of first to fourth instars larvae and pupae 687.14, 913.10, 1011.89, 1058.85 and 1 141.65 ppm, respectively[51]. In the present results, *E. hirta* leaf extract against first to fourth instars larvae and pupae of *An. stephensi* has been studied in the laboratory condition. The lethal concentrations (LC_{50}/LC_{90}) of *E. hirta* were 137.40,

172.65, 217.81, 269.37 and 332.39 ppm, respectively. The LC_{90} values of 470.69, 531.43, 590.77, 685.60 and 779.80 ppm, respectively.

The methanol leaf extract of *Calotropis gigantea* and bacterial insecticide, *B. thuringiensis* have mosquitocidal property was evaluated as target species of mosquito vectors[52]. This is an ideal eco-friendly approach for the control of vector control programs. In the present study, *B. sphaericus* at different concentrations brought out toxicity against the various larval instars malarial vector, *An. stephensi*. Similarly, the microbial pesticide spinosad against the malarial vector, *An. stephensi* showed 85% mortality. The observed mortality rate suggests that the above extract can be used as bio-pesticides. The LC_{50} of second, third and fourth instars larvae of *An. stephensi* were 0.27%, 0.28% and 0.30%, respectively[53-57]. Earlier, ten microbial products to develop a strategy to control mosquito larval and pupal population in the lab and field. Highest larval mortality was evident in the lab with LC_{50} and LC_{90} at 0.25 and 0.5 at 24 h for *Aedes aegypti*[58].

The toxicity of the wild-type *B. thuringiensis* subsp. *Bacillus israelensis*-H14 (Bti) and *B. sphaericus*-2362 (Bs) was determined towards *Aedes aegypti* larvae, theLC_{50} were estimated to be 0.094 and 1.18 μg/mL and LC_{90} to be 0.179 and 2.12 μg/mL, respectively and the Bti and Bs spore-crystal toxins were assayed in six different proportions that resulted in LC_{50} and LC_{90} varying from 0.018 to 1.51 μg/mL and 0.090 to 2.88 μg/mL, respectively[59]. Strains of *B. sphaericus* are known to have high activity towards larvae of *Culex*, variable toxicity to *Anopheles* depending on the species, and are inactive against Aedes larvae[60]. The larvicidal efficacy of SPH-88 against larvae of *Culex quinquefasciatus* is higher than that of the same larval stages of *Anopheles arabiensis*[61]. The formulated product of *B. sphaericus* BSN-0011 is effective against laboratory reared *Culex quinquefasciatus*. In our study the efficacy of *B. sphaericus* exhibited significantly higher toxicity on first instars than on the second instars larvae of *An. stephensi*[62].

Field trials on the efficacy of mosquito nets treated with a tablet formulation of deltamethrin (K-OTAB®) in Sundargarh District of Orissa state, India showed reduction in malaria incidence[63,64]. Efficacy of aqueous suspension of Bti (Vectobac 12 AS) was investigated in a laboratory and field conditions against *Anopheles culicifacies* and *An. stephensi* and found effective[65] Field trials were conducted at Anwona and Mmemiriwa villages located at Ghana on residual activity of deltamethrin-impregnated durable residual wall lining against susceptible *Anopheles gambiae* even 3 weeks after installation on both cement and mud surfaces, found 100% mortality on both surfaces using WHO cone bioassay kits[66]. More field studies are needed to establish the utility of these interventions at personal and household levels. Larvicidal activity of the emulsified neem oil formulation was observed against late instars of *An. stephensi* larvae in tanks and pits, and 100% reduction was found[67].

The field-tested relatively stable lipid-rich fractions of neem products were as effective as good quality crude neem products in the control of culicine vectors of *Japanese encephalitis* and produced a slight but significant reduction in population of anopheline pupae[68]. *Azadirachta* excels Jack showed excellent larvicidal properties at low concentrations against *Culex pipiens molestus*. Its LC_{50} value after 1 day was 62.5 μg/mL[69]. In our recent study, the field trials were conducted by using *Clerodendrum inerme* and *Acanthus ilicifolius* treatment in different habitats of three species of mosquito vectors namely *An. stephensi*, *Aedes aegypti*, *Culex quinquefasciatus* (Vadavalli, Mettupalayam, Navavoor privu, Pommanampalayam, Mettupalayam, Kallaru Ooty) in Tamil Nadu, India. The percentage reduction of larval mortality also showed the variations among the different breeding habitats of mosquito vectors at 24, 48 and 72 h. This may be due to the impact of geographical distribution of *An. stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* at the breeding sites[70]. Similarly, in the present study the combined activity of plant extract of *E. hirta* and *B. sphaericus* in the field were 44.23%, 81.64% and 100.00% at 24, 48 and 72 h, respectively. This results shows that *B. sphaericus* and EHLE pesticides can control the malarial vector, *An. stephensi*.

The current investigation revealed that the crude extract of *E. hirta* and *B. sphaericus* possesses remarkable mosquito properties against *An. stephensi* mosquitoes. This study is the first to report on the mosquito combined larvicidal and pupicidal activity *E. hirta* and *B. sphaericus*. These results show that these two biological agents could reduce the malarial incidence. Further studies are in progress to evaluate the effect of purified extract on larvicidal activity. The result shows that good larvicidal and pupicidal properties of against vector control programs.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Rahman SJ, Sharma SK, Rajagopal. *Manual on entomological surveillance of vector borne diseases*. New Delhi: NICD; 1989.

[2] Lee SE, Kim JE, Lee HS. Insecticide resistance in increasing interest. *Agric Chem Biotechnol* 2001; **44:** 105–112.
[3] Macedo ME, Consoli RAGB, Grandi TSM, Anjos AMG, Oliveira AB, Mendes NM, et al. Screening of Asteraceae (Compositae) plant extracts for larvicidal activity against *Aedes fluviatilis* (Diptera: Culicidae). *Mem Inst Oswaldo Cruz* 1997; **92:** 565–570.
[4] Ansari MA, Razdan RK, Tandon M, Vasudevan P. Larvicidal and repellent actions of *Dalbergia sissoo* Roxb. (F. Leguminoseae) oil against mosquitoes. *Bioresour Technol* 2000; **73:** 207–211.
[5] Sharma P, Mohan L, Srivastava CN. Phytoextract-induced developmental deformities in malaria vector. *Bioresour Technol* 2006; **97**(14): 1599–1604.
[6] Rasheed M, Afshan F, Tariq RM, Siddiqui BS, Gulzar T, Mahmood A, et al. Phytochemical studies on the seed extract of *Piper nigrum* Linn. *Nat Prod Res* 2005; **19**(7): 703–712.
[7] Siddiqui BS, Gulzar T, Mahmood A, Begum S, Khan B, Afshan F. New insecticidal amides from petroleum ether extract of dried *Piper nigrum* L. whole fruits. *Chem Pharm Bull (Tokyo)* 2004; **52** (11): 1349–1352.
[8] Sandeep BP, Nilofar SN, Chandrakant SM. Review on phytochemistry and pharmacological aspects of *Euphorbia hirta* Linn. *J Pharma Res Health Care* 2009; **1:**113–133.
[9] Anonymous. *Euphorbia hirta* L. [Online] Available from: http://florabase.calm.wa.gov.au/browse/profile/4629. 2008. [Access on 31 May 2010].
[10]Evans FJ, Soper CJ. The tigliane, daphnane and ingenane diterpenes, their chemistry, distribution and biological activities. *Lloydia* 1978; **41:** 193–233.
[11]Wititsuwannakul R, Wititsuwannakul D, Sakulborirug C. A lectin from the bark of the rubber tree (*Hevea brasiliensis*). *Phytochemistry* 1998; **47:** 183–187.
[12]O'Keefe BR. Biologically active proteins from natural product extracts. *J Nat Prod* 2001; **64:** 1373–1381.
[13]Gálvez J, Crespo ME, Jiménez J, Suárez A, Zarzuelo A. Antidiarrhoeic activity of quercitrin in mice and rats. *J Pharm Pharmacol* 1993; **45**(2): 157–159.
[14]Chen L. Polyphenols from leaves of *Euphorbia hirta* L. *Zhongguo Zhongyao Zazhi* 1991; **16**(1): 38–39.
[15]Iwu MM. *Handbook of African medicinal plants*. CRC, Boca Raton, FL; 1993, p. 24–33.
[16]Wei H, Hou Y, Yang G, You M. Repellent and antifeedant effect of secondary metabolites of non-host plants on *Plutella xylostella*. *Yingyong Shengtai Xuebao* 2004; **15**(3): 473–476.
[17]Inserra RN, Dunn RAM, Sorley R, Langdow KR, Richmer AY. Weed hosts of *Rotylenchulus reniformis* in ornamental nurseries of S. Florida. *Weed Abstr* 1989; **39:** 444.
[18]Adamu HM, Abayeh OJ, Agho MO, Abdullahi AL, Uba A, Dukku HU, Wufem BM. An ethnobotanical survey of Bauchi State herbal plants and their antimicrobial activity. *J Ethnopharmacol* 2005; **99:** 1–4.
[19]Parekh J, Chanda VS. *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants. *Turk J Biol* 2007; **31:** 53–58.
[20]Nirmal Sharma JS, Qadry B, Subramanium T, Verghese SJ, Rahman SK, Jalees S. Larvicidal activity of *Gliricidia sepium* against mosquito larvae of *Anopheles stephansi, Aedes aegypti* and *Culex quinquefasciatus*. *Pharmaceutical Biol* 1998; **36** (1): 3–7.
[21]Murugan K, Jeyabalan D. Effect of certain plant extracts against the mosquito, *Anopheles stephensi* Liston. *Curr Sci* 1999; **76:** 631–633.
[22]Mohan L, Sharma P, Srivastava CN. Comparative efficacy of *Solanum xanthocarpum* extracts alone and in combination with a synthetic pyrethroid, cypermethrin, against malaria vector, *Anopheles stephensi*. *Southeast Asian J Trop Med Public Health* 2007; **38**(2): 256–260.
[23]Rajkumar S, Jebanesan A. Oviposition deterrent and skin repellent activities of *Solanum trilobatum* leaf extract against the malarial vector *Anopheles stephensi*. *J Insect Sci* 2005; **5:** 15.
[24]Meisch MV. Evaluation of *Bacillus sphaericus* against *Culex quinquefasciatus* in septic ditches. *J Am Mosq Cont Assoc* 1990; **6:** 496–499.
[25]National Institute of Communicable Diseases (NICD). Proceedings of the National Seminar on operation research on vector control in filariasis. New Delhi; 1990.
[26]Poopathi S, Tyagi BK. Studies on *Bacillus sphaericus* toxicity related resistance development and biology in the filariasis vector *Culex quinquefasciatus* (Diptera: Culicidae) from South India. *Appl Entomol Zool* 2002; **37:** 365–371.
[27]Balaraman K, Balasubramanian M, Jambulingam P. Field trial of *Bacillus thuringiensis* H-14 (VCRC B-17) against *Culex* and *Anopheles* larvae. *Indian J Med Res* 1983; **77:** 38–43.
[28]Armengol G, Hernandez J, Velez JG, Orduz S. Long-lasting effects of a *Bacillus thuringiensis serovar israelensis* experimental tablet formulation for *Aedes aegypti* (Diptera: Culicidae) control. *J Econ Entomol* 2006; **99:** 1590–1595.
[29]Geetha I, Manonmani AM. Surfactin: a novel mosquitocidal biosurfactant produced by *Bacillus subtilis* sp. subtilis (VCRC B471) and influence of abiotic factors on its pupicidal efficacy. *Let Appl Microbial* 2010; **51**(4): 406–412.
[30]Kovendan K,Murugan K, Vincent S, Kamalakannan S. Larvicidal efficacy of *Jatropha curcas* and bacterial insecticide, *Bacillus thuringiensis*, against lymphatic filarial vector, *Culex quinquefasciatus* Say. (Diptera: Culicidae). *Parasitol Res* 2011a; **109:** 1251–1257.
[31]Kovendan K, Murugan K, Vincent S, Barnard DR. Studies on larvicidal and pupicidal activity of *Leucas aspera* Willd. (Lamiaceae) and bacterial insecticide, *Bacillus sphaericus* against malarial vector, *Anopheles stephensi* Liston. (Diptera: Culicidae). *Parasitol Res* 2012a; **110:** 195–203.
[32]Wirth MC, Yang Y, Walton WE, Federici BA. Evaluation of alternative resistance management strategies for *Bacillus sphaericus*. Mosquito Control Research, Annual Report. Division of Agriculture and Natural Resources. University of California; 2001.
[33]Vogel AI. *Textbook of practical organic chemistry*. London: The English Language Book Society and Longman; 1978, p. 1368.
[34]Abbott WS. A method of computing the effectiveness of an insecticide. *J Am Mosquito Contr* 1925; **3:** 302–303.
[35]Finney DJ. *Probit analysis*. London: Cambridge University Press; 1971, p. 68–78.
[36]Alder HL, Rossler EB. *Introduction to probability and statistics*. San Francisco: Freeman; 1977, p. 246.
[37]World Health Organization. *Global plan to combat neglected tropical diseases 2008–2015*. 2007; WHO/CDS/NTD/2007.40.
[38]Kager PA. Malaria control: constraints and opportunities. *Trop Med Int Health* 2002; **7:** 1042–1046.
[39]Van den Berghe G. Metabolic effects of fructose in the liver. *Curr Top Cell Regul* 1978; **13:** 97–135.
[40]Nisha M, Kalyanasundaram M, Paily KP, Abidha Vanamail P, Balaraman K. *In vitro* screening of medicinal plant extracts for

macrofilaricidal activity. *Parasitol Res* 2007; **100**(3): 575-579.

[41]Singhi M, Joshi V, Dam PK. Studies on *Calotropis procera* as larvicidal and repellent plant against vectors of dengue and DHF in Rajasthan, India. *Annual report 2005-06, 2006*. Jodhpur: Desert Medicine Research Center; p. 24-28.

[42]Wandscheer CB, Duque JE, da Silva MAN, Fukuyama Y, Wohlke JL, Adelmann J et al. Larvicidal action of ethanolic extracts from fruit endocarps of *Melia azedarach* and *Azadirachta indica* against the dengue mosquito *Aedes aegypti*. *Toxicon* 2004; **44:** 829-835.

[43]David JP, Rey D, Pautou MP, Meyran JC. Differential toxicity of leaf litter to dipteran larvae of mosquito developmental sites. *J Invertebr Pathol* 2000; **75:** 9-18.

[44]Maurya P, Mohan L, Sharma P, Batabyal L, Srivastava CN. Larvicidal efficacy of *Aloe barbadensis* and *Cannabis sativa* against the malaria vector *Anopheles stephensi* (Diptera: Culicidae). *Entomol Res* 2007; **37:** 153-156.

[45]Govindarajan M, Jebanesan A, Pushpanathan T. Larvicidal and ovicidal activity of *Cassia fistula* Linn. leaf extract against filarial and malarial vector mosquitoes. *Parasitol Res* 2008; **102:** 289-292.

[46]Tomass Z, Hadis M, Taye A, Mekonnen Y, Petros B. Larvicidal effects of *Jatropha curcas* L. against *Anopheles arabiensis* (Diptera: Culicidea). *MEJS* 2011; **3**(1): 52-64.

[47]Pandey V, Agrawal V, Sharma K, Raghavendra K, Dash AP. Micropropagation of a traditional medicinal plant, Akarkara (*Spilanthes acemella* L.) and its bioefficacy against malaria and filarial vectors *Anopheles stephensi* Liston and *Culex quinquefasciatus*. Proceeding of National symposium on plant Biotechnology: New Frontiers. CIMAP, Lucknow; 2005, p. 18-27.

[48]Sakthivadivel M, Daniel T. Evaluation of certain insecticidal plants for the control of vector mosquitos′ vis. *Culex quinquefasciatus, Anopheles stephensi* and *Aedes aegypti*. *Appl Entomol Zool* 2008; **43:** 57-63.

[49]Kalu IG, Ofoegbu U, Eroegbusi J, Nwachukwu CU, Ibeh B. Larvicidal activities of ethanol extract of *Allium sativum* (garlic bulb) against the filarial vector, *Culex quinquefasciatus*. *J Med Plants Res* 2010; **4**(6): 496-498.

[50]Amer A, Mehlhorn H. Persistency of larvicidal effects of plant oil extracts under different storage conditions. *Parasitol Res* 2006b; **99:** 473-477.

[51]Mahesh Kumar P, Murugan K, Kovendan K, Subramaniam J, Amerasan D. Mosquito larvicidal and pupicidal efficacy of *Solanum xanthocarpum* (Family: Solanaceae) leaf extract and bacterial insecticide, *Bacillus thuringiensis*, against *Culex quinquefasciatus* Say (Diptera: Culicidae) *Parasitol Res* 2012a; **110**: 2541-2500.

[52]Kovendan K, Murugan K, Prasanna Kumar K, Panneerselvam C, Mahesh Kumar P, Amerasan D, et al. Mosquitocidal properties of *Calotropis gigantea* (Family: Asclepiadaceae) leaf extract and bacterial insecticide, *Bacillus thuringiensis*, against the mosquito vectors. *Parasitol Res* 2012d; **111**: 531-544.

[53]Aarthi N, Murugan K. Larvicidal and repellent activity of *Vetiveria zizanioides* L, *Ocimum basilicum* Linn and the microbial pesticide spinosad against malarial vector, *Anopheles stephensi* Liston (Insecta: Diptera: Culicidae). *J Biopesticides* 2010; **3**(1): 199-204.

[54]Prabhu K, Murugan K, Nareshkumar A, Ramasubramanian N, Bragadeeswaran S. Larvicidal and repellent potential of *Moringa oleifera* against malarial vector, *Anopheles stephensi* Liston (Insecta: Diptera: Culicidae). *Asian Pac J Trop Biomed* 2011; **1**(2): 124-129.

[55]Govindarajan M, Mathivanan T, Elumalai K, Krishnappa K, Anandan A. Ovicidal and repellent activities of botanical extracts against *Culex quinquefasciatus, Aedes aegypti* and *Anopheles stephensi* (Diptera: Culicidae). *Asian Pac J Trop Biomed* 2011; **1**(1): 43-48.

[56]Aarthi N, Murugan K. Effect of *Vetiveria zizanioides* L. Root extracts on the malarial vector, *Anopheles stephensi* Liston. *Asian Pac J Trop Dis* 2012; **2**(2): 154-158.

[57]Maurya P, Sharma P, Mohan L, Verma MM, Srivastava CN. Larvicidal efficacy of *Ocimum basilicum* extracts and its synergistic effect with neonicotinoid in the management of *Anopheles stephensi*. *Asian Pac J Trop Dis* 2012; **2**(2): 110-116.

[58]Kamalakannan S, Murugan K. Laboratory and field evaluation of *Metarhizium anisopliae* for the control of dengue vector, *Aedes aegypti* (Insecta: Diptera: Culicidae). *Toxicol & Environmental Chem* 2011.

[59]Sreshty MA, Kumar KP, Murty US. Synergism between wildtype *Bacillus thuringiensis* subsp. *israelensis* and *B. sphaericus* strains: A study based on isobolographic analysis and histopathology. *Acta Trop* 2011; doi:10.1016/j.actatropica.2010.12.012.

[60]Ramoska WA, Singer S, Levy R. Bioassay of three strains of *Bacillus sphaericus* on field collected mosquito larvae. *J Inverte Pathol* 1977; **30:** 151-154.

[61]Seyoum A. Mosquito larvicidal *Bacillus strains* from Ethiopia. MSc. Thesis. Addis Ababa University. 1995.

[62]Mulla MS, Chaney JD. Evaluation of Bacillus sphaericus liquid concentrates formulation against *Culex* mosquitoes. In: March J, editor. *Mosquito control research*. California: University of California Press; 1994, p.19-21.

[63]Sharma SK, Tyagi PK, Padhan K, Upadhyay AK, Mohanty HMA, SS RK, et al. Epidemiology of malaria transmission in forest and plain ecotype villages in Sundargarh District, Orissa, India. *Trans R Soc Trop Med Hyg* 2006a; **100:** 917-925.

[64]Sharma SK, Upadhyay AK, Haque MA, Padhan K, Tyagi PK, Batra CP, et al. Effectiveness of mosquito nets treated with a tablet formulation of deltamethrin for malaria control in a hyperendemic tribal area of Sundargarh district, Orissa, India. *J Am Mosq Control Assoc* 2006b; **22**(1): 111-118.

[65]Mittal PK. Biolarvicides in vector control: challenges and prospects. *J Vec Borne Dis* 2003; **40:** 20-32.

[66]Stiles-Ocran JB, Knowles SP, Wilson MD, Boakye DA, Jhomas J. Field evaluation of Durable Residual Wall Lining (DL) as an alternative to Indoor Residual Spraying (IRS) for the control *Anopheles gambiae* in Rural villages of Obuasi, Ghana. 5th MIM Pan-African malaria conference, 2-6 Nov. 2009, Narobi, Kenya. Poster no. 653; 2009, p. 178.

[63]Dua VK, Pandey AC, Raghavendra K, Gupta A, Sharma T, Dash AP. Larvicidal activity of neem oil (*Azadirachta indica*) formulation against mosquitoes. *Malar J* 2009; **8:** 124.

[64]Rao DR, Reuben R, Nagasampagi BA. Development of combined use of Neem (*Azadirachta indica*) and water management for the control of culicine mosquitoes in rice fields. *Med Vet Entomol* 1995; **9:** 25-33.

[65]Mustafa MA, Al Khazaraji A. Effect of some plant extracts on the *Culex pipiens* molestus Forskal larvae. *Iraqi J Vet Sci* 2008; **22:** 9-12.

[66]Kovendan K, Murugan K. Effect of medicinal plants on the mosquito vectors from the different agro-climatic regions of Tamil Nadu, India. *Adv Environ Biol* 2011; **5**(2): 335-344.